METHOD 8327

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

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Disclaimer

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts formally trained in the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purposes of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

This method covers the analysis of selected per- and polyfluoroalkyl substances (PFAS) in prepared samples or sample extracts by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

The 24 PFAS that have been evaluated with this method are provided below. This method has been tested in surface water, groundwater, and wastewater matrices. Some precision and bias data are provided in Table 1 (Sec. 17.0). This determinative method may also be applicable to other PFAS target compounds and other matrices, provided that the laboratory can demonstrate adequate performance (refer to Sec. 9.0 or project-specific acceptance criteria) using representative sample matrices. Please refer to Method 8000 for additional information.

Analyte	Preparation Method 3512	CAS RN[†]
PFAS sulfonic acids		
Perfluoro-1-butanesulfonic acid (PFBS)	✓	375-73-5
Perfluoro-1-pentanesulfonic acid (PFPeS)	✓	2706-91-4
Perfluoro-1-hexanesulfonic acid (PFHxS)	✓	355-46-4
Perfluoro-1-heptanesulfonic acid (PFHpS)	✓	375-92-8
Perfluoro-1-octanesulfonic acid (PFOS)	✓	1763-23-1
Perfluoro-1-nonanesulfonic acid (PFNS)	✓	68259-12-1
Perfluoro-1-decanesulfonic acid (PFDS)	✓	335-77-3
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS)	✓	757124-72-4
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	*	27619-97-2

1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	✓	39108-34-4
PFAS carboxylic acids		
Perfluorobutanoic acid (PFBA)	✓*	375-22-4
Perfluoropentanoic acid (PFPeA)	✓*	2706-90-3
Perfluorohexanoic acid (PFHxA)	✓	307-24-4
Perfluoroheptanoic acid (PFHpA)	✓	375-85-9
Perfluorooctanoic acid (PFOA)	✓	335-67-1
Perfluorononanoic acid (PFNA)	✓	375-95-1
Perfluorodecanoic acid (PFDA)	✓	335-76-2
Perfluoroundecanoic acid (PFUnDA)	✓*	2058-94-8
Perfluorododecanoic acid (PFDoDA)	✓*	307-55-1
Perfluorotridecanoic acid (PFTrDA)	✓*	72629-94-8
Perfluorotetradecanoic acid (PFTeDA)	✓*	376-06-7
PFAS sulfonamides and sulfonamidoacetic acids		
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)	✓*	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)	✓*	2355-31-9
Perfluoro-1-octanesulfonamide (PFOSA)	✓	754-91-6

Performance data from a large multi-laboratory validation study were used to update this table (data can be found in Sec. 17.0, Table 1).

✓ Acceptable precision and bias can be obtained for this analyte with this preparation method.

✓* Acceptable precision and bias can be obtained for this analyte with this preparation method. However, this analyte may require special care to ensure analytical performance will meet the needs of the project. See Sec. 1.3 for specific information regarding this analyte.

* This analyte did not meet the criteria for acceptable performance using this preparation technique and determinative method and may require special care to ensure analytical performance will meet the needs of the project. See Sec. 1.3 for specific information regarding this analyte.

† Standards for some target analytes may consist of mixtures of structural isomers; however, the Chemical Abstracts Service (CAS) Registry Number (RN) listed in the table is for the linear isomer. All CAS RNs in the above table are for the acid form. Sulfonic acids in stock standard mixes are typically received as the sodium or potassium salt form. CAS RNs for the salt form are not included.

1.1 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600 and 8000) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and (ii) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this method is provided by the U.S. Environmental Protection Agency (EPA) as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.2 This method is restricted to use by, or under supervision of, appropriately experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.3 The following target compounds may require special treatment when being determined by this method:

1.3.1 During method development the following compounds showed a potential for loss either during standard preparation (resulting in low bias to calibration standards and high recoveries for samples) or during sample preparation (resulting in low recoveries). Extra care should be taken to ensure that the composition of the stock and intermediate standards (those above the high calibration standard) maintain a high enough proportion of organic cosolvent to limit loss from solution (See Sec. 7.4). Sub-sampling from aqueous sample containers prior to adding sufficient organic solvent will also result in a loss of these and potentially other compounds from solution, the extent of which will be container dependent (See Sec. 8.0).

Perfluoroundecanoic acid (PFUnDA)

Perfluorododecanoic acid (PFDoDA)

Perfluorotridecanoic acid (PFTrDA)

Perfluorotetradecanoic acid (PFTeDA)

N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)

N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)

1.3.2 The following compounds lack or have low abundance of secondary product ions, and interferences may make qualitative identification more difficult.

Perfluorobutanoic acid (PFBA).

Perfluoropentanoic acid (PFPeA)

Perfluorohexanoic acid (PFHxA)

Perfluoro-1-octanesulfonamide (PFOSA)

1.3.3 Background contamination must be carefully evaluated and managed to a level is acceptable for the project-specific data application. During validation of Method 3512, some laboratories had problems meeting quality control acceptance criteria for 1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS) due to high and/or sporadic background contamination. More information about identifying and minimizing sources of contamination is presented in Sec. 4.0.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared using an appropriate sample preparation method (e.g., solvent dilution or extraction). Prepared samples or extracts are then analyzed by LC/MS/MS using external standard calibration.

2.2 Target compounds are qualitatively identified in samples by comparing retention times (RTs) to RTs of isotopically labeled surrogates in the same samples or to RTs of target analytes in standards, as applicable, and by comparing product ion ratios to those in standards (Sec. 11.6, Sec. 17.0 Table 2). Qualitatively identified target compounds are then quantitated based on their primary product ion responses utilizing external standard calibration (Sec. 11.7).

3.0 DEFINITIONS

Refer to SW-846 Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure. See Glossary (Appendix A) for relevant terms and acronyms.

4.0 INTERFERENCES

4.1 In order to avoid compromising data quality, contamination of the analytical system by PFAS from the laboratory must be reduced to the lowest practical level. Method blanks (MBs) and reagent blanks (RBs) are prepared and analyzed with all samples and are used to demonstrate that laboratory supplies and preparation and analysis steps do not introduce interferences or PFAS artifacts at levels that would prevent the proper identification and integration of target analytes or bias quantitation, especially near the Lower Limit of Quantitation (LLOQ) or any project-specific concentration levels of interest. Careful selection of reagents and consumables is necessary because even low levels of PFAS contamination may alter the precision and bias of the method; background introduced by these materials (and variability thereof) is cumulative. See Sec. 9.5 for blank acceptance criteria. Refer to each method to be used for specific guidance on QC procedures and to SW-846 Chapter Four for general guidance on cleaning of reusable labware.

4.2 Refer to Methods 3500, 3600, and 8000 for discussions of interferences. Matrix interferences can be caused by contaminants from the sample, sampling devices, or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations of the sample matrix.

4.3 The following procedures are employed to minimize problems with measurement precision and bias.

4.3.1 All solvents should be LC/MS grade, or equivalent, to minimize interference problems. Solvents must be checked by lot prior to use.

4.3.2 PFAS contamination has been found in reagents, glassware, tubing, polytetrafluoroethylene (PTFE) LC vial caps, disposable pipets, aluminum foil, glass disposable pipettes, filters, degassers, and other apparatus that release fluorinated compounds. All supplies and reagents should be verified prior to use. If found, measures should be taken to remove the contamination, if possible, or find other suppliers or materials to use that meet method- or project-specified acceptance criteria.

4.3.3 The LC system used should have components replaced, where possible, with materials known to not contain PFAS target analytes of interest.

4.3.4 During method development, loss of some PFAS target analytes was observed during storage of standard solutions in 1:1 methanol-water containing 0.1% acetic acid in glass containers. Polypropylene containers should be used for preparation and storage of samples and standards. Other materials may be used, such as high density polyethylene (HDPE), if it can be shown the target analytes are not adversely affected (i.e., all quality control criteria in Sec. 9.0 can be met). Glass autosampler vials have been successfully used for solutions in 1:1 methanol-water containing 0.1% acetic acid during analysis.

4.3.5 An isolator column should be placed downstream of the solvent pumps and any mixer or degasser and before the sample injection valve to delay the elution of contaminants from the LC system to the analytical column.

4.3.6 If labware is re-used, the procedure described for labware cleaning (Sec. 6.2.4, or equivalent) should be followed to minimize risk of carryover contamination. The blank QC acceptance criteria in Sec. 9.5 can be used as a guideline for evaluating cleanliness.

4.4 Where measured analyte concentrations are suspected of being high-bias and/or false positive results due to contamination, the laboratory should inform the data user of any suspected data quality issues and qualify affected data appropriately.

4.5 High concentrations of the native 4:2 FTS, 6:2 FTS, and 8:2 FTS target analytes will interfere with the primary product ion signals listed in Sec. 17.0, Table 2 for the M2-4:2 FTS, M2-6:2 FTS and M2-8:2 FTS surrogates. This interference results from the natural abundance of the ^{34}S isotope (~4.2% abundance relative to ^{32}S) in the native FTS target analytes and can lead to high bias recovery of these surrogates. Using the secondary product ions identified in Table 2 for quantitation of these surrogates will minimize this interference because this

surrogate product ion loses the $^{13}C_2$-labeled fluorocarbon sidechain to produce $^{32}SO_3H^-$ (m/z 81), while the ^{34}S isotope of the target analyte produces $^{34}SO_3H^-$ (m/z 83) from the same nominal mass precursor ion.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of U.S. Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses.

5.2 Users of this method should operate a formal safety program.

5.3 The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. Exposure to these chemicals should be reduced to the lowest possible level, and the appropriate personal protective equipment (PPE) should be utilized. Review SDSs for specific physical and health hazards including appropriate PPE to be used. SDSs can be accessed at multiple locations (e.g., www.sigmaaldrich.com, www.well-labs.com, and www.isotope.com).

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those used during method development or subsequently evaluated by the Agency. Labware, reagents, supplies, equipment, and settings other than those listed in this method may be employed provided that method performance appropriate for the intended application has been demonstrated and documented, including meeting acceptance criteria for all categories of quality controls listed in Sec. 9.0. This section does not list all common labware (e.g., beakers and flasks) that might be used.

6.1 Equipment

6.1.1 Liquid chromatograph (LC) system: An ultra performance liquid chromatograph (UPLC®) with stainless steel flow through needle design was used to generate data during method development (PEEK needles may not puncture polyethylene caps; pre-slitting of caps is not allowed).

6.1.2 Analytical LC columns: The following columns were used to generate data during method development:

6.1.2.1 Acquity UPLC® CSHTM Phenyl-Hexyl, 2.1 x 100 mm and 1.7 µm particle size (Waters part no. 186005407)

6.1.2.2 ZORBAX RRHD Stable Bond C18, 2.1 x 100 mm and 1.8 µm particle size (Agilent part no. 858700-902)

6.1.2.3 Accucore RP 2.1 x 100 mm and 2.6 µm particle size (Thermo part no. 17626-102130)

6.1.2.4 Shim-pack SP-C18, 2.1 x 150 mm and 2.7 µm particle size (Shimadzu part no. 227-32003-04)

6.1.3 Isolator columns:

6.1.3.1 XBridge BEH C18, 2.1 x 50 mm and 3.5 µm particle size (Waters part no. 186003021)

6.1.3.2 ZORBAX RRHD Eclipse Plus C18, 50 × 3.0 mm, 1.8 µm (Agilent part no. 959757-302)

6.1.3.3 BDS HypersilC18, 2.1 x 50 mm and 5 µm particle size (Thermo part no. 28105-052130)

6.1.3.4 Shim-pack XR-ODS II, 2 x 75mm and 2.2 µm particle size (Shimadzu part no. 228-41605-93)

6.1.4 Tandem Mass Spectrometry (MS/MS) System: A mass spectrometer must be capable of MS/MS analysis with a cycle time sufficient to obtain at least ten mass spectra over each chromatographic peak. The system must be capable of documenting the performance of the MS/MS system against manufacturer specifications for mass resolution, mass assignment, and sensitivity using the internal calibrant (See Sec. 11). Sensitivity should be sufficient to meet project-specified needs in the matrices of interest, where practical (See Secs. 7.4.4.1 and 9.9). A triple quadrupole mass spectrometer with an electrospray ionization source was used to generate data during method development.

6.2 Support Equipment and Supplies

6.2.1 Adjustable volume pipettes, 10 µL to 10 mL with polypropylene tips.

6.2.2 Analytical balance, capable of weighing to 0.01 g for determining sample mass or to 0.0001 g for preparing standards from neat.

6.2.3 Sample containers and miscellaneous supplies; all supplies should meet blank criteria in Sec. 9.5 where practical.

6.2.3.1 Autosampler vials: HDPE, polypropylene or glass

6.2.3.2 Polyethylene autosampler vial caps (Waters Catalog # 186004169)

6.2.3.3 50-mL polypropylene tubes (BD Falcon, Catalog # 352098)

6.2.3.4 15-mL polypropylene tubes (BD Falcon, Catalog # 352097); pre-weighed tubes are recommended for collection of field samples and field QC

6.2.3.5 Polyethylene disposable pipettes (Samco Thermo Scientific, Catalog # 252)

6.2.3.6 Polypropylene pipette tips (Eppendorf, Catalog #s 022491997, 022492080, 022491954, 022491946, and 022491512)

6.2.4 Reusable labware cleaning instructions – If labware is re-used it should be washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. All labware is subsequently rinsed with organic solvent(s) such as acetone, methanol, and acetonitrile. Traces of target compounds should be reduced to a minimum.

7.0 REAGENTS AND STANDARDS

7.1 Chemicals used in all tests should be LC/MS grade if available, or reagent grade at a minimum. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be verified prior to use to ensure the blank acceptance criteria in Sec. 9.5 can be met.

7.2 Reagent water

All references to water in this method refer to reagent water as defined in SW-846 Chapter One, unless otherwise specified. Reagent water from in-house deionized water treatment systems may need additional treatment prior to use (e.g., with a point-of-use water purification system) to meet blank acceptance criteria (Sec. 9.5). Bottled reagent water should be evaluated in the same manner as reagent water from other sources.

7.3 Reagents and Gases

7.3.1 Acetonitrile, C_2H_3N (CAS RN 75-05-8)

7.3.2 Ultrapure argon and nitrogen

7.3.3 Methanol, CH_3OH (CAS RN 67-56-1)

7.3.4 Isopropyl alcohol, C_3H_8O (CAS RN 67-63-0)

7.3.5 Sodium hydroxide, NaOH (CAS RN 1310-73-2)

7.3.6 Ammonium acetate, $C_2H_7NO_2$ (CAS RN 631-61-8), neat

7.3.7 Glacial acetic acid, CH_3COOH (CAS RN 64-19-7)

7.4 PFAS standard solutions

The following sections describe preparation of stock solutions, spiking solutions, and calibration standards for the compounds of interest. This discussion is provided as an example. Other approaches and concentrations of the target compounds may be used if appropriate for

the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.4.1 Storage and Shelf-life

While not in use, store standard solutions at ≤6°C in the refrigerator or according to the manufacturer's recommended storage conditions. Bring standard solutions to room temperature and mix well prior to use. Keep standard solutions away from PFAS-containing packaging and materials.

Use the manufacturer's expiration date for purchased prepared standards and neat source materials, as applicable. The laboratory may develop QC practices for determining expiration dates for standards prepared from certified reference materials; otherwise, use one year from date of preparation for expiration of prepared standard solutions. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards or spiking solutions.

7.4.2 Stock standards

Solutions may be purchased as certified solutions or prepared from neat certified reference materials.

For standard solutions prepared from neat materials, the weight may be used without correction to calculate the concentration of the stock standard when standard compound purity is assayed to be 96% or greater. Commercially prepared stock standards may be used at any concentration if they are certified by an accredited supplier or third party.

NOTE: Esterification of fluorinated carboxylic acids in methanolic solutions is known to occur over time. If base is not already present, adding a small amount of strong base to stock standards will improve stability of these analytes in methanol. The Wellington stock standards listed below include 4 mole equivalents of sodium hydroxide (*i.e.*, 4 moles of OH^- per mole of PFAS target analytes). The equation below can be used to estimate an amount of sodium hydroxide to add, which uses an assumed molar mass of 250 g/mol and is reproduced from EPA Method 533 (Sec. 16.0 Reference 9).

$$\frac{Total\ PFAS\ mass\ (g) \times 160\ \left(\frac{g}{mol}\right)}{250\ \left(\frac{g}{mol}\right)} = Mass\ of\ NaOH\ Required\ (g)$$

7.4.2.1 Target compounds stock

A mixture of 24 target analytes from Wellington Laboratories was used for method validation (Catalog # PFAC-24PAR, 2000 ng/mL in methanol). See Table 1 for this list of target analytes. Sulfonic acids in this mixture were

prepared from salts, and some had certified concentrations of both linear and branched isomers.

NOTE: Correct concentrations of salt forms to acid (or base) concentrations for reporting purposes. For example, the certificate of analysis from a Wellington standard (PFAC-24PAR) included the concentration of PFBS as a potassium salt at 2000 ng/mL and as the acid at 1,770 ng/mL

7.4.2.2 Surrogates stocks

A mixture of 19 isotopically labeled surrogates from Wellington Laboratories was used for method validation (Catalog # MPFAC-24ES, 1000 ng/mL in methanol). See Sec. 17.0, Table 5 for this list of surrogates and suggested target analyte associations.

7.4.3 Spiking solutions

Spiking solutions should be prepared in 95:5 acetonitrile-water. Alternate solvents (e.g. 96:4 methanol-water) may be used provided that method performance is not adversely affected and the QC criteria in Sec. 9.0 can be met. The following sections have suggested spiking concentrations for 5 mL water samples that may be appropriate for use with Method 3512.

CAUTION: Loss of longer-chain PFAS from solution can occur at lower proportions of organic co-solvent.

7.4.3.1 PFAS target compounds spiking solution

PFAS target analytes (Sec. 7.4.2.1) are added to LLOQ verification, LCS and MS/MSD QC samples prior to preparation from the same source materials used to prepare ICAL standards. LCS and MS/MSD QC samples should be spiked at concentrations near the mid-point ICAL standard concentration after all sample preparation steps are complete, assuming 100% recovery. LLOQ verification QC samples should be spiked at concentrations near (0.5-2x) the established or anticipated LLOQ standard concentration after all sample preparation steps are complete, assuming 100% recovery.

Example preparation of a target compounds spiking solution for LCS and MS/MSD QC samples: A 100 µL aliquot of a stock PFAS target analytes mix at 2000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile-water produces a solution at 20 ng/mL concentration (nom.). Addition of 40 µL of this solution to 5 mL aqueous LCS and MS/MSD QC samples would result in target analyte concentrations of 160 ng/L.

Example preparation of target compounds spiking solution for LLOQ verification QC samples: A 10 µL aliquot of a stock PFAS target analytes mix at 2000 ng/mL concentration diluted to a final volume of 10 mL with 95:5 acetonitrile-water produces a solution at 2 ng/mL concentration (nom.). Addition

of 25 µL of this solution to a 5 mL aqueous LLOQ verification QC sample would result in target analyte concentrations of 10 ng/L.

7.4.3.2 PFAS surrogates spiking solution

Isotopically-labeled PFAS surrogates (Sec. 7.4.2.2) are added to field samples and associated QC samples prior to preparation using the same source materials as used to prepare ICAL standards. Surrogates should be spiked at near the mid-point ICAL standard concentration after all sample preparation steps are complete, assuming 100% recovery.

Example preparation of surrogates spiking solution: A 200 µL aliquot of a stock PFAS surrogates mix at 1000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile- water produces a solution at 20 ng/mL concentration (nom.). Addition of 40 µL of this solution to a 5 mL water sample would result in surrogate concentrations of 160 ng/L.

7.4.4 Calibration standards – Two types of calibration standards are used for this method: standards made from the primary source used for ICAL and continuing calibration verification (CCV), and standards made from a second source used for initial calibration verification (ICV). When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

NOTE: Both linear and branched isomers of some PFAS target analytes may be present in commercially available standards (e.g., PFHxS, PFOS, N-MeFOSAA, N-EtFOSAA), while others target analytes may be represented by only a linear isomer. Please see Sec. 11.3.3 for additional information.

7.4.4.1 ICAL

ICAL standards are recommended to be prepared using the same target analytes and surrogates spiking solutions used for sample preparation (See Sec. 7.4.3). These spiking solutions can be used to prepare a calibration standards stock that is further diluted to make individual calibration standards. A minimum of five different calibration standard concentrations is required for a linear (first-order) calibration model, and a minimum of six concentrations is required for a quadratic (second-order) model. Regardless of calibration model, the lowest ICAL standard concentration must be at or below the LLOQ (see Sec. 9.9 and Method 8000) and should be sufficient to meet any sensitivity DQOs identified for the project. The remaining standards should correspond to the range of concentrations found in actual samples but should not exceed the working range of the LC/MS/MS system. Each calibration standard should contain all the desired project-specific target analytes for which qualitative and quantitative results are to be reported by this method.

Example preparation of calibration standards stock solution: A 100 µL aliquot of a target compounds spiking solution at 20 ng/mL (nom., Sec. 7.4.3.1) and a 100 µL aliquot of a surrogates spiking solution at 20 ng/mL (nom., Sec.

7.4.3.2) brought to 10 mL with a 1:1 methanol-water solution containing 0.1% acetic acid would produce a solution of target analytes and surrogates at concentrations of 200 ng/L. This calibration standards stock can then be used to prepare lower concentration ICAL standards by diluting aliquots with appropriate volumes of 1:1 methanol-water containing 0.1% acetic acid.

NOTE: Acetic acid is added primarily because it improves chromatography for some target analytes.

NOTE: Calibration standards should not be reused once the caps are pierced unless the vial is immediately recapped. Volatile losses can occur rapidly because punctures of polyethylene caps leave large holes, and there are no septa to mitigate losses.

7.4.4.2 ICV: Second source standards for ICV are prepared using certified reference materials from a second manufacturer or from a manufacturer's batch prepared independently from the batch used for calibration. A second lot number from the same manufacturer may be adequate to meet this requirement. Target analytes in the ICV are recommended to be prepared at concentrations near the mid-point of the calibration range. The ICV standard must contain all calibrated target analytes that will be reported for the project, if readily available.

7.4.4.3 Continuing calibration verification (CCV): CCV standards should be prepared in the same manner as ICAL standards at concentrations near the middle of the calibration range.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation, and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in a regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation, and storage requirements.

8.1 Sample collection criteria – Grab samples are collected in polypropylene containers. Other types of container materials, such as high-density polyethylene (HDPE), may be used if performance is acceptable for the project. PTFE containers and contact surfaces with PTFE should be avoided. Depending on the needs of the project, field blanks may be required and should be collected according to recommended PFAS sampling practices, where available. The samplers should acquire pre-verified reagent water and containers from the analytical laboratory for preparing field blanks, where practical. Aqueous field samples and associated QC samples must be collected in separate containers, including field blanks, MS/MSDs, and duplicates. Volumes collected for water samples should match volumes consumed in the laboratory’s preparation procedure. Conventional laboratory practices

involving chain of custody, field sampling, laboratory custody beginning with receipt and transfer custody, and sampling protocols should be followed.

CAUTION: Surface binding of target compounds from aqueous solution to collection containers is known to occur. Subsampling or transfer of water from a container prior to addition of a sufficient proportion of organic solvent can result in significant loss of longer-chain PFAS target analytes (e.g., carboxylic acids ≥C_9, sulfonic acids ≥C_7). Aqueous samples and sample extracts containing significant amounts of water may only be subsampled or transferred to other containers if 50% organic co-solvent content is achieved beforehand. Quantitative transfer can be achieved by solvent-rinsing the empty container with methanol. If subsampling is performed prior to achieving 50% organic cosolvent content, i.e., when preparing the entire water sample is not possible or practical, the data must be qualified appropriately.

8.2 Sample preservation, storage and holding times

All samples are iced or refrigerated at ≤6 °C from the time of collection until sample analysis. In the laboratory, samples and sample extracts should be stored in the refrigerator at ≤6 °C while not being analyzed. Formal holding times have not yet been established for these analytes in various matrices. A 14-day limit from sample collection to preparation and a 30-day limit from preparation to analysis may be used as a guide until a more formal study is completed.

NOTE: There is some evidence that suggests freezing samples can prevent transformation of some PFAS analytes into others. Longer sample holding times may be appropriate for the PFAS target analytes in this method. See Sec. 16.0, References 1, 4, 8, and 9.

9.0 QUALITY CONTROL

9.1 General guidance – Refer to SW-846 Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving collection of analytical data should include development of a structured and systematic planning document, such as a quality assurance project plan (QAPP) or a sampling and analysis plan (SAP), which translates project objectives and specifications into directions for those implementing the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged. Procedures for handling QC failures and associated corrective actions should be defined in the laboratory's SOP or in project planning documents (e.g., QAPP, SAP). Refer to Method 8000 for more information and guidance on evaluation and reporting of sample data associated with non-compliant quality controls. All sample data files and QC data files should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 and 3600 for QC procedures to ensure the proper operation of sample preparation and cleanup techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3500, 3600, or 8000.

9.3 QC procedures necessary to evaluate the LC system operation are found in Sec.11.3 of this method and in Method 8000, including evaluation of RT windows, calibration verification, and chromatographic peak shapes in standards and samples.

9.4 Initial demonstration of proficiency (IDP) – An IDP must be performed by the laboratory prior to independently running an analytical method and should be repeated if other changes occur (e.g., significant change in procedure, change in personnel). Refer to Method 8000 Sec. 9.0 for additional information regarding instrument, procedure, and analyst IDPs. An IDP must consist of analysis of a minimum of four replicate reference samples for each sample preparation and determinative method combination utilized and by generating data of acceptable precision and bias for target analytes in a clean reference matrix taken through the entire preparation and analysis procedure.

9.5 Blanks

9.5.1 Before processing any samples, the analyst must demonstrate through the analysis of a MB or RB that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source of the contaminant peak and eliminate it, if possible. As a continuing check, each time a batch of samples is prepared and analyzed, and when there is a change in reagents, an additional MB must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. MBs and field blanks must be carried through all stages of sample preparation and analysis. At least one MB or RB must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples.

9.5.2 Blanks are generally considered to be acceptable if target analyte concentrations are less than one half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations/responses are ≥10X the blank). Other criteria may be used depending on the needs of the project.

9.5.3 If an analyte of interest is found in a sample in the batch near a concentration confirmed in the blank (refer to Sec. 9.5.2), the presence and/or concentration of that analyte should be considered suspect and may require qualification. Samples may require re-extraction and/or re-analysis if the blanks do not meet laboratory-established or project-specific criteria. Re-extraction and/or re-analysis is *not* necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.5.4 When new reagents or chemicals are received, the laboratory should monitor the blanks associated with samples for any signs of contamination. It may be necessary to test every new batch of reagents or chemicals prior to sample preparation as PFAS contamination is common. If reagents are changed during a preparation batch, separate blanks should be prepared for each set of reagents.

9.5.5 The laboratory should not subtract the results of the MB from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the MB results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the MB results, and the data qualified appropriately.

9.5.6 A minimum of one MB for every 20 field samples must be prepared in a blank matrix to investigate for PFAS contamination throughout sample preparation, extraction, and analysis.

9.5.7 A minimum of one RB should be analyzed daily and is prepared with a 1:1 methanol-water solution containing 0.1% acetic acid to investigate for system/reagent contamination. PFAS contamination at low levels is common in laboratory supplies and equipment. RBs are prepared and analyzed to help identify potential sources of contamination. Surrogates are not required to be added to RBs.

9.6 Sample QC for preparation and analysis

The laboratory must have procedures for documenting the effect of the matrix on method performance (precision, bias, sensitivity). At a minimum, this must include preparation and analysis of a MB and LCS, and where practical, an MS/MSD pair or MS and duplicate in each preparation batch, as well as monitoring the recovery of surrogates. An LLOQ verification QC sample is recommended to be included in each sample preparation batch, as needed for the project. All reported target analytes are recommended to be included in LLOQ verification, LCS and MS/MSD QC samples. Any MBs, LLOQ verifications, LCSs, MS/MSDs, and duplicate samples should be subjected to the same sample preparation and analysis procedures as those used on actual samples (See Sec. 11.0 and any relevant sample preparation and cleanup methods). Consult Method 8000 for more details on QC procedures for sample preparation and analysis, including information on developing statistically based acceptance criteria for sample preparation QC.

9.6.1 Matrix Spikes/Duplicates

Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one MS/MSD pair. The decision on whether to prepare and analyze an MS and duplicate or a MS/MSD pair should be based on knowledge of the samples and addressed in project planning documents. If samples are expected to contain target analytes, laboratories may use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, then laboratories should use a MS/MSD pair. Project defined acceptance limits are recommended for MS/MSD % recovery and MS/MSD or sample/duplicate relative % difference (RPD); statistically derived acceptance limits may be used in the

absence of project specifications.

9.6.1.1 When sufficient sample is available, MS/MSDs are prepared for each matrix at a minimum frequency of one MS/MSD pair for every 20 field samples to investigate for matrix interferences.

9.6.1.2 As part of a QC program, recovery of target analytes is monitored for each matrix type. Bias is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the MS, MSD (if performed), LCS, and any LLOQ verifications according to the following formula.

$$Recovery = \%R = \frac{(C_s - C_u)}{C_n} \ x \ 100$$

where:

C_s = Measured concentration of spiked sample aliquot
C_u = Measured concentration of unspiked sample aliquot (use 0 for LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS or LLOQ verifications).

NOTE: MS/MSD recoveries may not be meaningful if the amount of analyte in the sample is large relative to the amount spiked.

9.6.1.3 A duplicate sample or MSD is analyzed with every batch of 20 field samples, where available. The relative percent difference (RPD) between the sample and duplicate or MS and MSD should be ≤30% (or less than statistically derived acceptance limits or project defined acceptance limits). A laboratory control sample duplicate (LCSD) may be used to evaluate precision in the batch if extra field sample containers are not received for performing duplicates or MSD.

Calculate the RPD between the MS and MSD or sample and duplicate using the following equation:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \ X \ 100$$

where:

C_1 =Measured concentration of first sample aliquot
C_2 =Measured concentration of second sample aliquot.

NOTE: Using approximately the same sample size or scaling the spike amount to the

sample size for the MS/MSD will minimize bias in the RPD calculation. See Method 8000 for more information.

9.6.2 LCS

LCS QC samples are prepared at a minimum frequency of one per batch of 20 or fewer field samples. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume, like the MB. The LCS is spiked with the same analytes and at the same concentrations as the MS/MSD, near the midpoint of the initial calibration range, when appropriate, and is taken through all sample preparation steps. When the results of the MS/MSD analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. See Sec. 9.6.1.2 for recovery calculation and to Sec 9.6.1.3 for RPD calculation if LCSD data are acquired. Preliminary acceptance criteria for LCS recovery are 70-130% recovery and ≤30% RPD. Statistically derived acceptance limits or project defined acceptance limits are recommended.

9.6.3 Surrogates

Surrogates are added to all field samples and associated QC samples as described in Sec. 11. An isotopically labeled structural analog of each target analyte is recommended, if available. If an isotopically labeled surrogate of sufficient purity cannot be obtained, target analytes should be associated with surrogates that are as chemically similar as possible. See Sec. 17.0, Table 5 for examples of surrogates and suggested target analyte associations. Preliminary acceptance criteria are 70-130% recovery. Statistically derived acceptance limits or project defined acceptance limits are recommended.

9.6.4 LLOQ verification

LLOQ verification QC samples are recommended to be included at a frequency of one per batch of 20 or fewer field samples, as needed for the project. Refer to Sec. 9.9 for more information about LLOQ verifications. These QC samples are taken through all sample preparation steps like MB and LCS samples. Preliminary acceptance criteria are 50-150% recovery. Statistically derived acceptance limits or project defined acceptance limits are recommended. Refer to Sec. 9.6.1.2 for recovery calculation.

9.7 Initial Calibration Acceptance Criteria (ICAL) – The LC/MS/MS system must be calibrated as described in Sec. 11.3. Prior to analyzing samples, verify the ICAL standards using a second source ICV standard, if readily available (See Sec. 7.4.4.2).

9.8 CCV – ICAL of the LC/MS/MS system must be verified using the procedure and at the frequency described in Sec. 11.4.

9.9 Lower Limits of Quantitation (LLOQs)

General guidance for verifying LLOQs is provided in this section and in Method 8000. The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence. The LLOQ must be

≥ the lowest point in the calibration curve. The laboratory shall establish LLOQs at concentrations where both quantitative and qualitative requirements can consistently be met (see below and Sec. 11.6). The laboratory shall verify the LLOQ at least annually by matrix and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at or near established LLOQs. LLOQ verifications are also recommended on a project-specific basis (Sec. 9.6.4). Optimally, LLOQs should be less than the desired decision levels or regulatory action levels based on stated project-specific DQOs.

NOTE: If project-specific concentration levels of interest are sufficiently high, the LCS may be sufficient to evaluate bias.

9.9.1 Verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.9.2 LLOQ verifications are prepared by spiking a clean control material with the analyte(s) of interest at 0.5 - 2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5 - 2 times the established or anticipated LLOQ. LLOQ verifications are carried through the same preparation and analytical procedures as environmental samples and other QC samples.

9.9.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Preliminary acceptance criteria for the LLOQ verification are 50-150%. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Statistically based LLOQ verification acceptance criteria should be determined once sufficient data points have been acquired.

9.9.4 Reporting concentrations below LLOQ – Concentrations that are below the established LLOQ may still be reported; however, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analyte concentrations reported below the LLOQ should meet the qualitative identification criteria in Sec. 11.6.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. Specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample preparation methods that have been validated in conjunction with this determinative method are listed in Sec. 1.0.

11.2 Sample cleanup – Cleanup procedures should not be necessary for relatively clean sample matrices. Extracts from highly contaminated environmental, waste or biota samples may require additional cleanup steps prior to analysis to meet acceptance criteria for all QC categories. The specific cleanup procedure used will depend upon the analytes of interest, the nature of the interferences, and the DQOs for the project. At the time of publication, no cleanup methods have been validated in conjunction with this determinative method.

11.3 ICAL

11.3.1 Tune the mass spectrometer according to the manufacturer's specifications after major repair or maintenance to the system or when mass shifts > 0.2 Dalton. Acceptable system performance may be demonstrated by meeting manufacturer specifications for mass resolution, mass accuracy, and sensitivity using an internal calibrant. Other performance measures may be appropriate for some projects. Initial calibration must not begin until performance criteria are met.

Optimize other instrument settings as needed to obtain acceptable performance for all chromatographic peaks and monitored product ions. LC/MS/MS conditions used in method development are listed in Sec. 17.0, Tables 3 and 4. Chromatographic peaks should be inspected to ensure they are symmetrical, and significant peak tailing should be corrected.

11.3.2 Analyze initial calibration standards across a range of concentrations appropriate for the sensitivity and linear range of the instrument or as needed for the project. Quantitation is based on external standard calibration models, with a minimum of five standards at different concentrations for average calibration factor or linear (first-order) calibration models, and a minimum of six standards for a quadratic (second-order) model. The lowest calibration standard must be at or below the LLOQ (see Sec. 9.9). See Sec. 11.4 in Method 8000 for additional information and guidance for initial calibration. Standards and samples must be analyzed under the same LC/MS/MS conditions, including injection volume.

NOTE: Visually inspect target analyte peaks in the calibration standard at the LLOQ to ensure that peak signal is adequately distinguishable from background and meets the qualitative identification criteria outlined in Sec. 11.6. Product ions used for quantitation should have signal to noise (S/N) ≥10, and any product ions used to support qualitative identification should have S/N ≥3.

11.3.3 Identify target compounds using the optimized product ion responses from Sec. 11.3.1. A secondary product ion is identified for most of the target analytes (Sec. 17.0, Table 2).

NOTE: PFAS target analytes may be represented by both linear and branched isomers (e.g. PFHxS, PFOA, PFOS, N-MeFOSAA, and N-EtFOSAA) which can be calibrated using a summation of the responses for all of the isomer peaks if present in quantitative standards (for example, sum or integrate all of the C6 sulfonic acid linear and branched isomers as one calibration point) or by calibrating with only the linear isomer. If a quantitative standard containing both linear and branched isomers is not available, a separate qualitative standard may be used to identify retention times of isomer peaks. Quantitative results should only be reported for peaks that are also identified in quantitative or qualitative standards, and a quantitative standard must be used for calibration. The data must be reported such that the calibration and quantitation choices are clear to the data user. See Figure 1, Sec. 17.0, for examples of chromatograms with linear and branched isomer peaks. For a more detailed explanation of one approach see Method 533.

11.3.4 Initial calibration models and acceptance criteria

Average calibration factor, linear, or quadratic regression models may be used for initial calibration. Options for evaluation of initial calibration fit are presented in the following subsections and can be applied independently. Alternate acceptance criteria may be applied depending on the needs of the project; however, those criteria should be clearly defined in a laboratory SOP or a project planning document (e.g., QAPP, SAP, or equivalent).

11.3.4.1 Average Calibration Factor (CF) calibration model

Relative Standard Deviation (RSD) of calibration factors should be ≤20%. Refer to Sec. 11.5.1 of Method 8000 for calculations. The average CF should not be used for compounds that have an RSD >20%.

11.3.4.2 Linear or quadratic regression models

Correlation coefficient (r) should be ≥0.995 or Coefficient of Determination (COD; r^2) should be ≥0.99. For regression calculations see Sec. 11.5.2 in Method 8000. % Error (Sec. 11.3.4.3) should also be evaluated when r or r^2 is used as an ICAL acceptance criterion, especially near the LLOQ. Weighted regressions can improve calibration fit and reduce % error especially at lower ICAL standard concentrations.

11.3.4.3 % Error

Percent error between the calculated concentration of each calibration standard and its expected (prepared) concentration should be ≤50% at the LLOQ and ≤30% at higher concentrations. Refer to Sec. 11.5.4.1 of Method 8000 for calculations.

11.3.4.4 Relative Standard Error (RSE)

RSE should be ≤20%. Refer to Sec. 11.5.4.2 of Method 8000 for calculations.

11.3.5 When the calibration does not meet the acceptance criteria, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

NOTE: It is considered inappropriate once the calibration models have been finalized to select an alternate fit solely to meet QC acceptance criteria for samples on a case-by-case basis.

11.3.6 When the ICAL acceptance criteria are not met, qualify the affected data, or refer to Sec. 11.5.6 of Method 8000 for recommended corrective actions. If more than 10% of the target compounds included with the ICAL (or more than 10% of those that will be reported) do not meet the established ICAL acceptance criteria (Sec. 11.3.4), then the system is considered unacceptable for sample analysis to begin. Correct the source of the problem and repeat the calibration procedure beginning with Sec. 11.3. If the problem persists, more in-depth troubleshooting may be necessary.

11.3.7 ICV – Prior to analyzing samples, verify the ICAL using a standard obtained from a second source to the calibration standard (see Sec. 7.4.4.2). Suggested acceptance criteria for the analyte concentrations in this standard are 70 - 130% of the expected analyte concentration(s). Alternative acceptance criteria may be appropriate based on project-specific DQOs. Quantitative sample analyses should not proceed for those analytes that do not meet the ICV criteria. However, analyses may continue for those analytes that do not meet the criteria with an understanding that these results could be used for screening purposes and qualified appropriately.

11.4 Continuing Calibration Verification (CCV)

11.4.1 Verify the initial calibration by analyzing a mid-level CCV standard prior to any samples, after every 20 field samples as needed (or every 12 hours, whichever is shorter), and at the end of the analytical sequence. The CCV is prepared from the same stock solutions or source materials used for the ICAL standards. The results must be calculated with the most recent ICAL and should meet the acceptance criteria provided below.

NOTE: A CCV may be omitted from the beginning of the analysis sequence if samples are analyzed within 12 hours of ICAL, and the injection of the last ICAL standard may be used as the starting time reference for evaluation.

11.4.2 CCV Acceptance Criteria

The calculated concentration or amount of each analyte of interest in the CCV standard should fall within ±30% of the expected value, which is equivalent to percent

difference (%D) or percent drift ≤±30%. Refer to Sec. 11.7 of Method 8000 for %D and % drift calculations.

11.4.3 When the CCV acceptance criteria are not met, qualify the affected data, or refer to Sec. 11.7 of Method 8000 for guidance. Due to the number of compounds that may be analyzed by this method, some compounds may fail to meet the acceptance criteria. The analyst should strive to place more emphasis on meeting the acceptance criteria for those compounds that are critical to the project. If the criterion is not met (i.e., %D or % drift >±30%) for more than 10% of the compounds included in the ICAL (or more than 10% of those that will be reported), then corrective action must be taken prior to analysis of samples, e.g., by analyzing a separately prepared CCV, or reanalyzing a CCV or new initial calibration after performing instrument maintenance.

NOTE: The analyst must closely monitor responses and chromatography in the CCVs for signs that the system is unacceptable for analysis to continue (e.g., unusual tailing, loss of resolution). If significant losses of target analytes/surrogates occur (e.g., <50% recovery, other laboratory-defined criteria) or if significant degradation of chromatography occurs, corrective action must be taken prior to sample analysis, or the analyst must demonstrate there is adequate sensitivity to meet project objectives.

11.4.4 A MB or RB must be analyzed after the ICAL or CCV and prior to samples to ensure that the system (i.e., introduction device, transfer lines and LC/MS system) is free from levels of contaminants that would bias the results. If the blank indicates contamination, then it may be appropriate to analyze additional blanks to help determine the source of contamination. A MB or RB is not required after a CCV at the end of an analytical sequence. Refer to Sec. 9.5.2 regarding qualification of data and/or corrective actions related to MB or RB contamination.

NOTE: Background of PFAS target analytes may increase in some LC systems while they are held under initial conditions or while idle; re-started sequences should typically begin with at least one blank to bleed out any accumulated background and to provide information about the potential for any carryover in the system. Refer to Sec. 9.5 for associated acceptance criteria.

11.5 Sample analysis procedure

11.5.1 Analyze samples using the same LC/MS/MS conditions as used to generate the ICAL. Warm samples to room temperature and mix well prior to transferring to vials.

A suggested sequence order is:

RB
ICAL standards and ICV, or opening CCV
MB
LLOQ verification
LCS
Field samples (with a CCV followed by a MB or RB every 20 field samples or 12 hours,

whichever is shorter)
Duplicates
Matrix spike/matrix spike duplicate
Closing CCV

11.5.2 The laboratory must monitor recoveries of isotopically-labeled surrogates (Sec. 7.4.2.2). The percent recovery of each surrogate should fall within the acceptance criteria, especially for QC samples prepared in clean matrices like reagent water (e.g., MB, LCS, LLOQ verification). If multiple surrogates fail to meet the acceptance criteria and/or the target analytes associated with the failing surrogate(s) are important to the project, reanalysis and/or repreparation of samples may be warranted. Otherwise, the associated target analytes may be reported with appropriate data qualifiers. See additional guidance in Sec. 9.6 of Method 8000.

11.5.3 If the concentration of any target analyte exceeds the ICAL range of the system, the prepared sample or sample extract should be diluted with 1:1 methanol-water containing 0.1% acetic acid and reanalyzed. If dilutions cannot be performed, concentrations that exceed the calibration range must be qualified as estimated. When the response of a compound in the sample exceeds the calibration range, analysis of a RB can help determine the extent of any carryover that may occur under the conditions used at the laboratory. See the caution after Sec. 8.1 about ensuring organic solvent content is sufficient prior to subsampling.

11.6 Qualitative identification of target analytes – Target analytes are qualitatively identified by comparison of relative responses of primary and secondary product ions in a sample to standards and by comparison of RT in a sample to the isotopically labeled surrogate in the same sample and/or to the target analyte in standards.

11.6.1 Identify target analytes by comparing the relative responses of primary and secondary product ions in the sample to those in a standard. Secondary product ions are identified for most target analytes (Sec. 17.0, Table 2). The primary/secondary (or secondary/primary) product ion ratio should be within ±50% of the average of the ion ratios in the initial calibration standards or the ion ratio in in the mid-level ICAL standard or preceding CCV, as defined in the laboratory's SOP. The analyst should use professional judgment when interferences are observed or when ion ratios are not met to avoid misidentification.

NOTE: Depending on sensitivity and matrix interference issues, a secondary product ion response might be used for quantitation rather than a primary product ion. Clearly identify any changes to analyte quantitation to the end data user.

NOTE: The primary and secondary product ion ratios and RTs in samples may not match calibration standards as well if samples contain different proportions of branched and linear isomers. Figure 1 (Sec. 17.0) shows how relative abundances of branched and linear isomers can differ in samples and standards, which may lead to differences in product ion ratios. The complete isomer grouping present in standards must be integrated consistently in all samples. Refer to Sec. 11.3.3 for more information.

11.6.2 RTs of target analytes should fall within ±0.1 min of its isotopically labeled analog in the same sample, if present. Otherwise, RTs of target analytes in samples should fall within ±0.2 minutes of the RT of the same target analyte in the mid-level ICAL standard, average of the ICAL standards, or the preceding CCV, as defined in the laboratory's SOP. Alternatively, a relative RT deviation (in %) may be used for confirmation of target compounds. Qualitative identification of target analytes without isotopically labeled analogs may also be supported by standard additions (e.g., MS/MSD). RT shifts may result in peaks eluting outside the analytical time segment in which the characteristic product ions are monitored, which could produce false negative results. Differences in relative proportions of linear and branched isomers in samples can also complicate evaluation of RT shift, and the laboratory should take care to avoid misidentification of peaks. Time segments and RT windows must include branched isomers, where applicable.

11.7 Analyte quantitation – Once a target compound has been identified, the quantitation of that compound will be based on the integrated abundance of the primary product ion unless interference problems are observed. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually, particularly for integration of linear and branched isomers. Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g., RT updates, integration parameter files, maintaining chromatographic peak shapes, etc.). The analyst should seek to minimize manual integration where practical by properly maintaining the instrument, updating RTs, and configuring peak integration parameters.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations and documentation – Sample concentrations are quantitated using the following equations:

$$Concentration\ in\ \frac{ng}{L} = \frac{(X_s)(V_t)(D)}{(V_s)}$$

$$Concentration\ in\ \frac{ng}{g} = \frac{(X_s)(V_t)(D)}{(W_s)}$$

where:

V_t = Total volume of extract or diluted sample (in L).
V_s = Volume of aqueous sample prior to preparation (in L).
D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. This value is always dimensionless.

W_s = Weight of sample extracted (in grams). If kg units are used for this term, multiply results by 1000 g/kg. Unless otherwise requested, report results in solids on a dry-weight normalized basis.

X_s = Calculated concentration of analyte (ng/L) from the analysis. Type of calibration model used determines derivation of X_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3 of Method 8000.

12.2 See Secs. 11.5 and 11.10 of Method 8000 for additional information and formulas for quantitating results.

13.0 METHOD PERFORMANCE

Please refer to Sec. 17.0, Table 1 for a summary of method performance from a multi-laboratory validation study of aqueous samples prepared by Method 3512.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult Less is Better: Laboratory Chemical Management for Waste Reduction, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety at: https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* (Sec. 16.0).

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables, figures, and appendices referenced by this method.

TABLE 1. RECOVERY AND PRECISION OF TARGET ANALYTES IN MULTI-LABORATORY STUDY MATRICES PREPARED BY METHOD 3512[1]

200 ng/L (nominal) prepared concentration												
	Reagent Water			Groundwater			Surface Water			Wastewater		
Target Analyte	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	96.4	10.6	16.1	97.2	5.8	11.0	90.2	9.7	16.0	95.4	12.7	14.7
PFPeA	100	4.6	13.1	100	5.2	11.3	99.5	3.8	11.9	102	6.0	13.8
PFHxA	99.5	8.2	8.5	96.9	9.2	6.1	98.9	7.6	7.4	98.7	12.5	7.3
PFHpA	101	6.4	8.4	98.8	7.1	5.7	100	5.9	6.4	100	9.3	6.4
PFOA	104	7.9	9.3	102	9.8	7.8	103	6.7	8.3	104	8.0	8.8
PFNA	104	7.0	10.1	101	8.1	8.1	101	8.1	11.2	103	8.9	9.0
PFDA	106	14.3	9.3	102	9.4	7.2	104	9.8	9.3	106	11.6	8.9
PFUnDA	105	8.3	14.8	103	8.6	16.4	103	7.7	12.4	106	10.0	18.7
PFDoDA	103	7.7	13.9	103	5.6	20.4	101	9.3	18.3	101	10.7	17.8
PFTrDA	103	11.2	15.7	99.3	8.4	16.6	99.4	7.5	17.2	98.2	14.1	16.2
PFTeDA	101	12.6	15.7	92.9	12.0	12.6	94.1	7.9	14.9	91.8	13.1	13.9
PFBS	100	9.3	9.4	101	8.5	6.7	99.2	7.3	9.0	99.5	10.0	7.8
PFPeS	99.0	6.2	7.4	99.2	4.7	7.4	98.7	4.2	8.4	100	7.3	6.7
PFHxS	101	6.9	8.3	101	5.6	6.0	101	6.0	6.5	106	13.8	9.0
PFHpS	104	4.9	10.1	102	5.0	8.9	100	6.6	9.1	104	6.4	10.1
PFOS	104	5.8	11.2	105	6.6	11.1	103	6.4	9.4	120	51.9	31.8
PFNS	106	7.6	13.9	106	5.8	14.3	106	7.5	12.2	104	9.4	14.7
PFDS	104	7.2	15.2	102	6.4	16.1	100	6.1	15.9	95.5	15.1	17.5
PFOSA	92.1	4.2	10.0	97.3	4.0	10.0	94.2	4.0	6.9	98.6	4.9	10.9
FtS 4:2	104	6.5	8.3	97.6	6.3	11.4	101	10.4	11.5	106	10.0	18.6
FtS 6:2	92.3	17.0	23.5	97.1	77.4	44.8	86.2	11.9	28.6	93.9	11.0	31.5
FtS 8:2	109	11.1	11.1	105	10.3	10.5	107	12.8	12.1	118	13.4	19.7
NMeFOSAA	100	11.6	9.2	103	12.9	14.1	103	13.2	14.9	100	15.4	11.4
NEtFOSAA	104	15.7	18.3	108	11.8	21.4	105	8.7	19.1	109	9.3	20.2

	60 ng/L (nominal) prepared concentration											
	Reagent Water			Groundwater			Surface Water			Wastewater		
Target Analyte	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)	$\bar{X}$[2] (%)	S_w[3] (%)	S_b[4] (%)
PFBA	93.1	13.1	19.6	98.0	15.6	17.9	86.7	16.4	21.4	96.6	14.6	16.0
PFPeA	104	13.5	13.7	113	30.7	34.9	103	10.2	16.6	107	11.8	11.6
PFHxA	97.4	11.7	8.2	95.0	13.7	7.1	98.2	16.9	16.6	98.2	14.8	17.3
PFHpA	98.5	8.6	10.7	96.2	11.2	8.3	95.6	12.5	10.4	101	7.8	8.4
PFOA	100	11.4	9.7	98.8	11.4	9.6	101	14.2	9.8	100	11.3	7.8
PFNA	97.0	9.6	8.9	96.5	12.7	11.2	95.1	10.9	11.4	98.5	12.0	6.9
PFDA	102	16.6	14.4	98.2	16.4	14.0	97.2	14.0	11.3	100	17.5	8.8
PFUnDA	98.9	13.2	14.7	96.0	12.3	13.2	96.3	16.4	13.1	98.2	15.1	14.3
PFDoDA	95.8	17.5	17.9	98.0	15.4	22.7	93.9	12.3	17.0	95.6	14.9	25.6
PFTrDA	97.7	14.0	19.3	95.8	11.8	23.1	92.3	14.4	19.7	97.2	20.0	21.3
PFTeDA	95.5	15.1	22.6	88.2	13.9	18.6	83.8	13.5	16.1	90.7	17.4	25.7
PFBS	92.7	11.3	4.9	99.1	15.9	6.8	94.9	13.3	8.2	100	11.1	8.6
PFPeS	96.8	8.3	6.2	95.8	7.1	7.8	95.3	10.5	9.8	96.3	8.4	3.6
PFHxS	95.3	10.8	12.9	94.9	12.3	14.5	96.9	9.0	11.4	102	9.7	11.7
PFHpS	101	11.5	9.5	97.5	11.8	10.9	96.5	10.9	10.5	100	11.3	9.2
PFOS	100	15.8	14.0	103	13.6	14.3	104	13.8	10.8	108	14.3	15.3
PFNS	102	9.9	14.9	99.8	10.2	17.0	99.4	9.8	18.2	101	11.7	14.2
PFDS	97.7	12.2	14.6	95.6	9.2	18.3	94.4	13.8	17.6	95.1	14.5	16.5
PFOSA	87.7	8.2	8.4	89.6	7.7	10.9	85.2	10.2	13.2	92.4	6.2	8.2
FtS 4:2	98.5	15.9	6.5	91.0	16.6	13.2	92.2	12.4	13.7	98.7	16.9	15.7
FtS 6:2	85.5	32.9	32.0	75.6	17.8	21.2	130	363	153	88.4	23.2	27.7
FtS 8:2	105	12.6	9.6	101	19.4	12.1	93.9	14.9	13.8	111	17.4	19.1
NMeFOSAA	98.6	16.8	10.5	96.0	16.1	13.4	98.5	22.6	20.9	99.8	20.2	13.3
NEtFOSAA	96.5	18.3	17.2	97.6	20.9	22.4	99.0	18.8	20.7	109	13.8	25.4

[1]% Recovery of each replicate sample was calculated after subtracting the mean unspiked concentration (n=5) by matrix determined at each laboratory

[2]Pooled mean % recovery (n=40 samples, including 5 replicates of each matrix and spike level tested by each of 8 laboratories except for 60 ng/L wastewater where n=39 samples); For calculation refer to Appendix G Section 3.2.4 (1) of "Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program, February 2018", available at: https://www.epa.gov/sites/production/files/2018-03/documents/chemical-new-method-protocol_feb-2018.pdf.

[3]Within-laboratory standard deviation of % recovery (n= 8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above

[4]Between-laboratory standard deviation of % recovery (n=8 laboratories); Refer to calculation in Appendix G Section 3.2.4 (1) in the reference above.

TABLE 2. RECOMMENDED PRECURSOR IONS AND PRODUCT IONS AND EXAMPLE CHROMATOGRAPHIC RETENTION TIMES

Target Analyte	Precursor Ion m/z	Product Ion m/z		Retention Time (min)[1]
		Primary	Secondary	
PFBA[2]	213	169	-	3.07
PFPeA[2]	263	219	-	4.16
4:2 FTS	327	307	81	5.13
PFHxA	313	269	119	5.46
PFBS	299	80	99	5.72
PFHpA	363	319	169	6.39
PFPeS	349	80	99	6.74
6:2 FTS	427	407	81	6.81
PFOA	413	369	169	7.08
PFHxS[3]	399	80	99	7.45
PFNA	463	419	219	7.68
8:2 FTS	527	507	81	7.92
PFHpS	449	80	99	8.08
PFDA	513	469	219	8.2
N-MeFOSAA	570	419	483	8.22
N-EtFOSAA	584	419	483	8.43
PFOS[3]	499	80	99	8.6
PFUnDA	563	519	269	8.7
PFNS	549	80	99	9.15
PFDoDA	613	569	169	9.17
PFTrDA	663	619	169	9.66
PFDS	599	80	99	9.67
PFOSA[2]	498	78	-	9.77
PFTeDA	713	669	169	10.12
Isotopically labeled surrogates[4]:				
M4PFBA	217	172		3.06
M5PFPeA	268	223		4.15
M2-4:2 FTS[5]	329	309	81	5.12
M5PFHxA	318	273		5.44
M3PFBS	302	80		5.72
M2PFHpA	367	322		6.38
M2-6:2 FTS[5]	429	409	81	6.8
M8PFOA	421	376		7.08
M3PFHxS	402	80		7.46
M9PFNA	472	427		7.66
M2-8:2 FTS[5]	529	509	81	7.92
M6PFDA	519	474		8.18
d3-NMeFOSAA	573	419		8.21
d5-NEtFOSAA	589	419		8.41
M8PFOS	507	80		8.63
M7PFUnDA	570	525		8.68
M2PFDoDA	615	570		9.17
M8PFOSA	506	78		9.77
M2PFTeDA	715	670		10.12

[1]RTs are based on conditions in Table 3 using the binary gradient.

[2]Secondary product ions with sufficient relative abundance were not identified for these chemicals using the conditions described in Tables 3 and 4.

[3]Branched isomers of PFHxS, PFOS, and potentially other perfluoroalkyl sulfonates may produce lower responses for product ions other than m/z 80, which may lead to low bias measurement of these isomers if used for quantitation.

[4]Secondary product ions are not identified for most isotopically labeled surrogates but may be useful.

[5]Using the m/z 81 product ion for quantitation of M2-4:2 FTS, M2-6:2 FTS, and M2-8:2 FTS will reduce interferences from high concentrations of the respective native target analytes compared to the primary product ions. Refer to Sec. 4.5 for more information.

TABLE 3. EXAMPLE LIQUID CHROMATOGRAPHY CONDITIONS

Analytical column: See Sec. 6.1.2.1

Isolator Column: See Sec. 6.1.3.1

Column temperature: 35-50ºC

Injection volume: 10-30µL

Needle wash: 60% acetonitrile / 40% 2-propanol

Binary Gradient

Time (min)	Flow rate (mL/min)	% Solvent Line A (20mM Ammonium Acetate in water)	% Solvent Line B (Acetonitrile)
0	0.3	100	0
1	0.3	80	20
6	0.3	50	50
13	0.3	15	85
14	0.4	0	100
17	0.4	0	100
18	0.4	100	0
21	0.4	100	0

Ternary Gradient:

Time (min)	Flow rate (mL/min)	% Solvent Line A (95:5 water-acetonitrile)	% Solvent Line B (Acetonitrile)	% Solvent Line C (400mM ammonium acetate in 95:5 water-acetonitrile)
0	0.3	95	0	5
1	0.3	75	20	5
6	0.3	50	45	5
13	0.3	15	80	5
14	0.4	0	95	5
17	0.4	0	95	5
18	0.4	95	0	5
21	0.4	95	0	5

TABLE 4. EXAMPLE MASS SPECTROMETER CONDITIONS

Instrument	Waters Xevo TQ-S
Ion Source	Electrospray Ionization (Negative mode)
Capillary voltage	0.75 kV
Source temp	150°C
Desolvation gas temp	450°C
Desolvation gas flow	800 L/hr
Cone gas flow	200 L/hr
Collision gas flow	0.15 mL/min
Collision energy	Optimized by analyte
Cone voltage	Optimized by analyte

TABLE 5. EXAMPLE SURROGATE AND TARGET ANALYTE ASSOCIATIONS

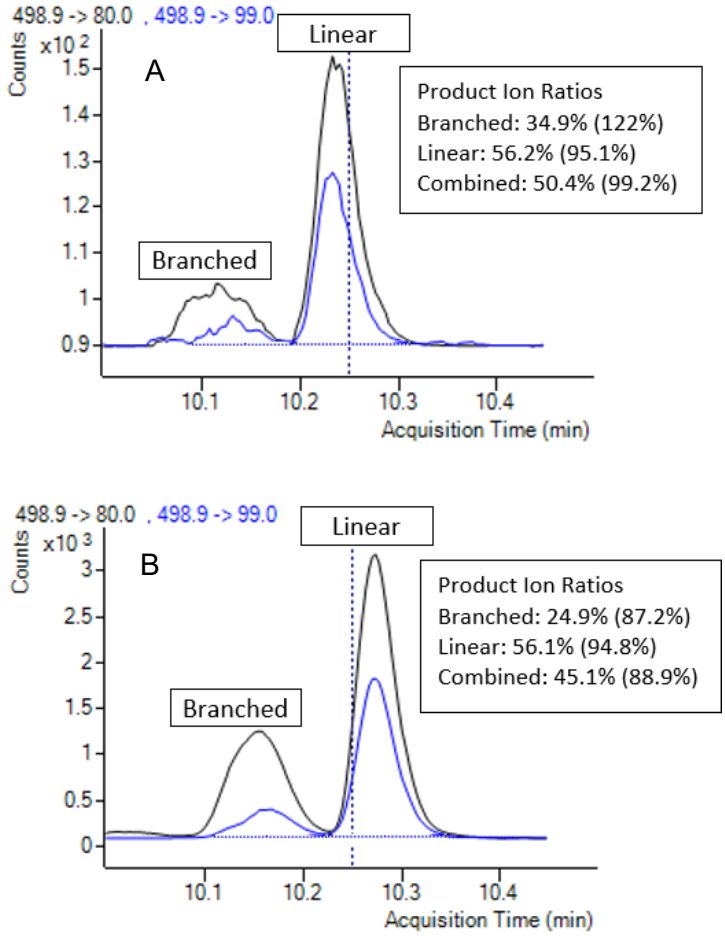
Examples of Isotopically Labeled PFAS Surrogates	Recommended target analyte association(s)
Sulfonic Acid Surrogates	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid (M3PFBS)	PFBS, PFPeS
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid (M3PFHxS)	PFHxS, PFHpS
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid (M8PFOS)	PFOS, PFNS, PFDS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] hexanesulfonic acid (M2-4:2 FTS)	4:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] octanesulfonic acid (M2-6:2 FTS)	6:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] decanesulfonic acid (M2-8:2 FTS)	8:2FTS
Carboxylic Acid Surrogates	
Perfluoro-n-[$^{13}C_4$]butanoic acid (M4PFBA)	PFBA
Perfluoro-n-[$^{13}C_5$]pentanoic acid (M5PFPeA)	PFPeA
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid (M5PFHxA)	PFHxA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid (M4PFHpA)	PFHpA
Perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA)	PFOA
Perfluoro-n-[$^{13}C_9$]nonanoic acid (M9PFNA)	PFNA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid (M6PFDA)	PFDA
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid (M7PFUnDA)	PFUnDA
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (M2PFDoDA)	PFDoDA, PFTrDA
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid (M2PFTeDA)	PFTeDA
Sulfonamide and Sulfonamidoacetic acid Surrogates:	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide (M8PFOSA)	PFOSA
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid (d3-N-MeFOSAA)	N-MeFOSAA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA)	N-EtFOSAA

TABLE 6. QC SUMMARY

Quality control category	Specification and minimum frequency	Acceptance criteria
Sample preservation, storage and holding time (Sec. 8.2)	Ice or refrigerate to ≤6ºC from sample collection to preparation; Recommended holding time from sample collection to preparation: 14 days Recommended holding time from preparation to analysis: 30 days	Use judgment to qualify data; formal holding time study pending
Initial Calibration (ICAL) (Sec. 9.7, 11.3)	Prior to analysis of samples	Mean CF: RSD ≤20% Linear or quadratic regression: r ≥0.995 or r^2 ≥0.99 %Error: ≤±50% at LLOQ and ≤±30% for higher concentrations RSE ≤20% ≥90% of target analytes and surrogates meet ICAL acceptance criteria
Initial calibration verification (ICV) (Sec. 9.7, 11.3.7)	After initial calibration and prior to analysis of samples	Target analytes are within ±30% of expected concentrations
Continuing calibration verification (CCV) (Sec. 9.8, 11.4)	Prior to analysis of field samples (unless ICAL analyzed in prior 12 hr), after every 20 samples and at end of sequence	≥90% of target analytes and surrogates within ±30% of expected concentrations
Reagent Blank (RB) (Sec. 9.5.7, 11.4.4)	One per day of analysis	Target analyte concentrations <1/2 LLOQ or <10% of sample concentrations
Method Blank (MB) (Sec. 9.5.6, 11.4.4)	One per preparation of 20 or fewer samples	Target analytes <1/2 LLOQ or <10% of sample concentration
Matrix spike/duplicate or matrix spike/matrix spike duplicate (MS/MSD) (Sec. 9.6.1)	One set per preparation of 20 or fewer field samples (if sufficient replicate samples are provided)	Meets laboratory derived or project specific recovery and RPD criteria
Laboratory Control Sample (LCS) (Sec. 9.6.2)	One per preparation batch of 20 or fewer samples	Within 70-130% recovery or within laboratory derived or project specific recovery criteria
Surrogates (Sec. 9.6.3)	Added to all field samples and prepared QC samples (MB, LLOQ Verification, LCS, MS/MSD)	Within 70-130% recovery or within laboratory derived or project specific recovery criteria

Quality control category	Specification and minimum frequency	Acceptance criteria
LLOQ verification (Sec. 9.6.4, 9.9.1)	Required annually. Recommended one per preparation batch of 20 or fewer samples	Within 50-150% recovery or within laboratory derived or project specific recovery criteria
Qualitative identification of target analytes (Sec. 11.6)	Evaluate each target analyte in field samples	RT in sample is within ±0.1 min of isotopically-labeled analog of target analyte, or otherwise within ±0.2 min of target analyte RT in midpoint ICAL standard, average of ICAL standards, or preceding CCV. For target analytes with secondary product ions, ratio of primary/secondary or secondary/primary product ion is within ±50% of expected ratio from midpoint ICAL standard, average of ICAL standards, or preceding CCV

FIGURE 1. PFOS PRIMARY AND SECONDARY PRODUCT IONS



NOTE: Product ion traces above show proportions of primary and secondary product ions for linear and branched PFOS isomers in (A) a calibration standard and (B) a field sample. Product ion ratios show the proportion of secondary product ion peak area to primary product ion peak area (in %), and the percentage in parentheses is the ratio in this standard or sample divided by the ratio in a reference standard. Refer to Sec. 11.3.3 for more information regarding integration of linear and branched isomer peaks in standards and samples and to Sec. 11.6.1 for more information regarding evaluation of product ion ratios to support qualitative identification.

APPENDIX A - GLOSSARY

ASTM	ASTM International, formerly American Society for Testing and Materials
CAS RN	Chemical Abstract Service Registry Number®
CCV	continuing calibration verification
DQOs	data quality objectives
EPA	U.S. Environmental Protection Agency
HDPE	high density polyethylene
ICAL	initial calibration
ICV	initial calibration verification
IDP	initial demonstration of proficiency
LC	liquid chromatography
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
LLOQ	lower limit of quantitation
MB	method blank
MS	matrix spike
MS/MS	tandem mass spectrometry – The process of separating precursor ions by m/z, followed by collisionally activated dissociation of a precursor ion at a given m/z into one or more product ions of smaller m/z.
MSD	matrix spike duplicate
m/z	mass-to-charge ratio
OSHA	U.S. Occupational Safety and Health Administration
PEEK	polyetheretherketone
PFAS	per- and polyfluoroalkyl substances
PPE	personal protective equipment
Precursor ion	Ion produced in the ion source that forms particular product ions or undergoes specified neutral losses during MS/MS analysis.
Product ion	Ion formed as the product of a reaction involving a particular precursor ion. See reference 9 in Section 16.0.
PTFE	polytetrafluoroethylene
QA	quality assurance
QAPP	quality assurance project plan

QC	quality control
RB	reagent blank
RSD	relative standard deviation
RT	retention time
SAP	sampling and analysis plan
SDS	safety data sheet
SOP	standard operating procedure
UPLC	Ultra Performance Liquid Chromatograph®